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Phenolic compounds with antioxidant capacity of the native Andean potato (*Solanum tuberosum* L.) Huagalina variety in La Libertad- Peru

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Abstract

Potato is grown and consumed throughout the world for their particular calorific nutritional value and vitamin C, but it also contains phytochemicals (secondary metabolites) that have been studied and found to have positive effects in preventing degenerative diseases in human health, such as hypertensive activity, and also atherosclerosis, type 2 diabetes, liver fibrosis, Alzheimer's disease, macular degeneration and cancer. We aimed to identify and quantify phenolic compounds in potatoes grown on El Zuro (EZ) and Huayatan Alto (HA) in Santiago de Chuco, La Libertad, Peru. The field trials were carried out in EZ (altitude 3750 m.a.s.l.) and HA (altitude 3150 m.a.s.l.) employing organic and inorganic fertilization respectively. Extraction from the peel and flesh was obtained separately, with the following solution: 50% methanol, 50% deionized water and 0.5% acetic acid. The sample was injected into the system UPLC - MS / MS, using ESI ionization (*Electrospray Ionization*) and fifteen external reference standards. Thirteen metabolites were detected in the flesh and potato peel. The highest content of secondary metabolites (mg/100 g DW) were: Chlorogenic acid (476.82 ± 63.58), neochlorogenic acid (87.90 ± 19.42) caffeic acid (77.53 ± 14.49) and vanillin (11.52 ± 1.38). The PCA (*Principal Components Analysis*) scores show that the highest concentration of metabolites was found in the peel of both cultivars. We concluded that the native potato Huagalina contains the genes expressed in different biosynthetic pathways of the metabolites found in this study.

Key words: native potato, phytochemicals, cancer, diabetes, chlorogenic acid.

1. Introduction

Solanum tuberosum L. is one of the most important crops, which originated in the Peruvian Andes and is widely consumed worldwide (Bordoloi *et al.*, 2012). According to FAO, world production of potatoes in 2012 was led by China (85.86E+06 t) and India (45E+06 t), Peru is in 18th place with 4.47E+06 t (INEI, 2014). La Libertad (in the north of Peru) with 4.08E+05 t of potato crop yield now ranks fourth in the national production (INEI, 2014); it has a diversity of natural conditions giving the region an important biological wealth which generates comparative advantages in the highlands over 3000 m above sea level where the native Huagalina potato, also called "Amarilla del Norte", is grown. Potato is the only major food crop that is a tuber, and is a food recognized as a good source of protein, carbohydrates, vitamin C, vitamin B6 and vitamin B3 and certain minerals such as potassium, phosphorus and magnesium (André *et al.*, 2009). It also contains phytonutrients or bioactive compounds which are the largest groups of secondary metabolites that occur as a response to the environment due to their high adaptability to grow in very diverse environments (Hawkes, 1990). Because of their beneficial effects on health (Navarre, 2009; Monro and Mishra, 2009) they are as important as phenolics, flavonoids, folate, anthocyanins, poly-amines and carotenoids (Ezequiel *et al.*, 2013), which are considered as antioxidants, protecting against reactive oxygen species (ROS)

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when consumed through diet (Virgili and Scacini, 2003; Kris-Etherton *et al*., 2004). The bioavailability of these metabolites in potatoes seems to have benefits for human health, in anti-hypertensive activity, prevention of atherosclerosis, type 2 diabetes, Alzheimer's disease, cancer (Zhao and Moghadasian, 2010); there is also evidence of their inhibitory effect against HIV (Tamura *et al*., 2006), cataracts and macular degeneration (Chuah *et al*., 2008) and of antimicrobial properties (Muthuswamy and Ruspasinghe, 2007). As a therapeutic alternative, potatoes are also used empirically by the population for problems of the gastric mucosa, which shows that fresh potatoes and tocosh (an andean preservation technique) have an antioxidant defense and cytoprotective effect on the gastric mucosa (Sandoval-Vegas *et al.*, 2010; Sandoval *et al.*, 2015). There are many studies that have been conducted to determine antioxidant capacity and total phenolic compounds following *in vitro* protocols; the results are inconsistent for the validation of the conclusions; the main disadvantage of these assays is that they only give an estimation of antioxidant capacity and total phenolic content (Sharma and Bhat, 2009; Ignat *et al*., 2011).

Dixon *et al*. (2006) argue that biological systems are extremely complex, and that phytonutrients of vegetables produced in response to the environment are secondary metabolites, which reveal processes of gene expression, action of gene products, and resulting metabolic networks that contribute to the remarkable diversity inherent in living systems. In this sense, metabolomics emerges as a relatively new approach to improve our understanding of these networks and help determine the biochemical composition of plants and other biological organisms, thus, the aim of this research was to identify and quantify fifteen phenolic compounds in the peel and flesh of the native Huagalina potato with UPLC-MS/MS.

2. Materials and methods

2.1 Chemical materials

HPLC grade standards were obtained from Sigma-Aldrich (USA): 3,5-dihydroxy-benzoic acid, neochlorogenic acid, chloro-genic acid, 4-hydroxybenzoic acid, caffeic acid, syringic acid, vanillin, *p*-coumaric acid, ferulic acid, sinapic acid, 4-hydroxy-3-methoxy-cinnamaldehyde, coumarin, daidzein, genistein, 7-hydroxyflavone.

2.2 Plant materials

Field trials were carried out in Santiago de Chuco - La Libertad at two experimental sites: El Zuro (EZ) and Huayatan Alto (HA).

- El Zuro (3 750 m.a.s.l.), soil with sandy loam texture, good drainage, moderate and deep slope, acid with a pH of 5.3, electrical conductivity: 0.3 dS /m (no salt), organic matter: 6.4% (very high), phosphorus 17 ppm (high) and potassium 477 ppm (very high), annual average temperature 9 °C (dry, cold climate) and organic manuring (Guano de Islas 2 t/ha) under drought conditions.
- Huayatan Alto (3 450 m.a.s.l.), loamy soil and good drainage, with sloping slightly pronounced and moderately deep, acid with a pH 5.0, electrical conductivity 1.5 dS/m (no salt), organic matter: 5.2% (high), phosphorus 14 ppm (average) and potassium 80 ppm (low), the average annual temperature of 10 °C (temperate climate with presence of drizzles) and inorganic fertilization (180-200-160 kg of NPK/ha, under drought conditions.

Samples of harvested potatoes from EZ and HA were washed with tap water and allowed to dry. They were then manually cut into slices of 5 mm thickness, freeze - dried and packaged with liquid nitrogen in sealed jars. They were transported by air to the Núcleo de Biomoleculas (NuBioMol) of the Federal University of Viçosa (Minas Gerais, Brazil).

2.3 Extraction

The freeze-dried slices were carefully skinned with a scalpel to separate the peel from the flesh, and ground separately to obtain a fine powder. The following extraction solution (ES) was prepared: 50% methanol (HPLC grade - Sigma Aldrich), 50% deionized water and 0.5% acetic acid (Fluka Analytical HPLC-grade). Phenolic compounds were extracted from the peel and flesh separately, and used the ES described by Narváez-Cuenca *et al*., (2012). Each 0.02 g of peel powder and 0.02 g of flesh power were extracted separately with 500 µL of the ES. The samples were mixed in vortex (VWR Analog Vortex Mixer, USA) 4 times for 10 seconds, sonicated (Branson Ultrasonic bath 3800, USA) for 10 min at 4 °C and kept in ice (30 minutes). After centrifugation (Eppendorf 5424, Germany) 15000 g for 10 minutes at 4 °C, 350 µL of the supernatant was removed and put in a new tube. The process was repeated two additional times with the resultant pellet and the supernatants were pooled. A final centrifugation (Eppendorf 5424, Germany) 20000 g for 10 min at 4 °C was performed to remove any remaining tissue suspension, and stored at -80 °C until further analysis. The pellets were discarded.

2.4 UPLC-MS/MS analysis

The sample was automatically injected (5 µl) in the system using UPLC (Agilent 1200 Infinity Series, USA) coupled to a Mass Spectrometry type triple Quadrupole (QqQ), (model 6430 Agilent Technologies, USA). Chromatographic separation was carried out on a column Zorbax Eclipse Plus C18 (1.8 µm, 2.1 x 50 mm) (Agilent, USA) in series with a guard column Zorbax SB-C18, 1.8 µm (Agilent, USA). The solvent used was: (A) acetic acid 0.02% in water and (B) acetic acid 0.02% in acetonitrile (LC-MS grade, Fluka Analytical). The solvent flow rate was 0.3 ml/min in a column temperature of 30 °C. A linear gradient with the following solvent proportions of solvent B was used: gradient from 0 to 11 minutes, 5 to 60% of B; 11 to 13 min 60 to 95% B; 13 to 17 minutes 95% of B; 17 to 19 min and 95 to 5% B; 19 to 20 minutes, 5% B. The ionization method used in the mass spectrometry was an ESI (*Electrospray Ionization*) following these conditions: gas temperature of 300 °C, nitrogen flow rate of 10 L/min, nebulizer pressure of 35 psi and capillary voltage of 4000 V. The equipment was operated on mode MRM (*Multiple Reaction Monitoring*).

The mass of the established precursor ion/fragment (m/z) was monitored using fragmentation tests for each molecule: 3,5 dihydroxybenzoic acid (155.02/137.01), chlorogenic acid (355.00/163.00), 4-hydroxybenzoic acid (139.12/121.00), 4-hydroxy-3-methoxycinnamaldehyde (179.10/147.04), coumarin (147.06/91.00), daidzein (255.00/199.00), genistein (271.00/243.00), 7-hydroxyflavone (239.07/137.00), neochlorogenic acid (353.10/179.00), caffeic acid (179.00/135.00), syringic acid (197.00/121.200), vanillin (151.00/92.00), *p*-coumaric acid (163.04/119.00), ferulic acid (193.00/134.00), sinapic acid (223.00/164.10). The first eight were scanned in positive mode and the last ones in negative mode. A calibration curve (from 0.1 ng to 100 µg) using the respective standards of each metabolite was generated to determine the absolute quantification. The generated data were analyzed using "MassHunter Workstation" VB 06.00 software to obtain the peak areas for each phenolic metabolite and the results were expressed in mg/100 g of dry tissue.

2.5 Statistical analysis

The Principal Components Analysis *(PCA*) was performed using XLSTAT 2015 Software (Digital license).

3. Results and discussion

Phenolic compounds found in the native Huagalina potato grown in EZ and HA are presented in Figure 1, and those with higher concentration (expressed in mg/100 g DW) are: Chlorogenic acid (476.82 ± 63.58 and 457.88 ± 140.46), neochlo-

rogenic acid (50.08 ± 30.53 and 87.90 ± 19.42), caffeic acid (76.50 ± 9.32 and 77.53 ± 14.48) and vanillin (11.52 ± 1.38 and 10.41 ± 2.58) respectively**.**

The contents in chlorogenic acid (CGA) and vanillin were on average higher at 3.97% and 9.63% respectively, on potatoes grown in EZ when compared to those from HA, while neochlorogenic and caffeic acids were lower in EZ in 43% and 1.34% than those from HA. The lower amount in EZ could be explained because neochlorogenic acid is an isomer of CGA, caffeic acid has probably been esterified by quinic acid to CGA, and both give the highest concentration for CGA in EZ.

The average values (mg/100 g DW) of CGA, caffeic acid and neochlorogenic were 467.34; 68.98 and 77.00 respectively for both experimental sites, higher than those reported by André *et al.* (2009) for native potatoes grown in Huancayo and Huancani, Department of Junin (Peru) for the same metabolites (441.2; 7.1; 5.8) and those reported by Narváez-Cuenca *et al.*, (2012) for Victory potato purchased in supermarkets in Wageningen - The Netherlands (25.43; 2.41; 1.16).

In this research the highest concentration of phenolic compounds was obtained for CGA (5-0 caffeoylquinic acid) and neochlorogenic acid (3-0 caffeoylquinic acid), and caffeic acid (trans-caffeic acid) giving a total of 613.32 mg /100 g DW.

This makes the native Huagalina potato a health-promoting agent as CGA acid and caffeic acid have been reported as inhibitors of carcinogenesis (Huang *et al*., 1998, Sancho and Pastore, 2012, Serafim *et al*., 2015), reducing obesity (Cho *et al*., 2010) hypotensive activity, providing cardiovascular protection (Susuki *et al*., 2002; Jiang *et al*., 2016).

Shi *et al*. (2016) showed that a new mechanism of CGA acid mediated in the protection of liver fibrosis (a stage before cirrhosis), inhibits oxidative stress and increases antioxidant defense and could become a promising new treatment for liver disease.

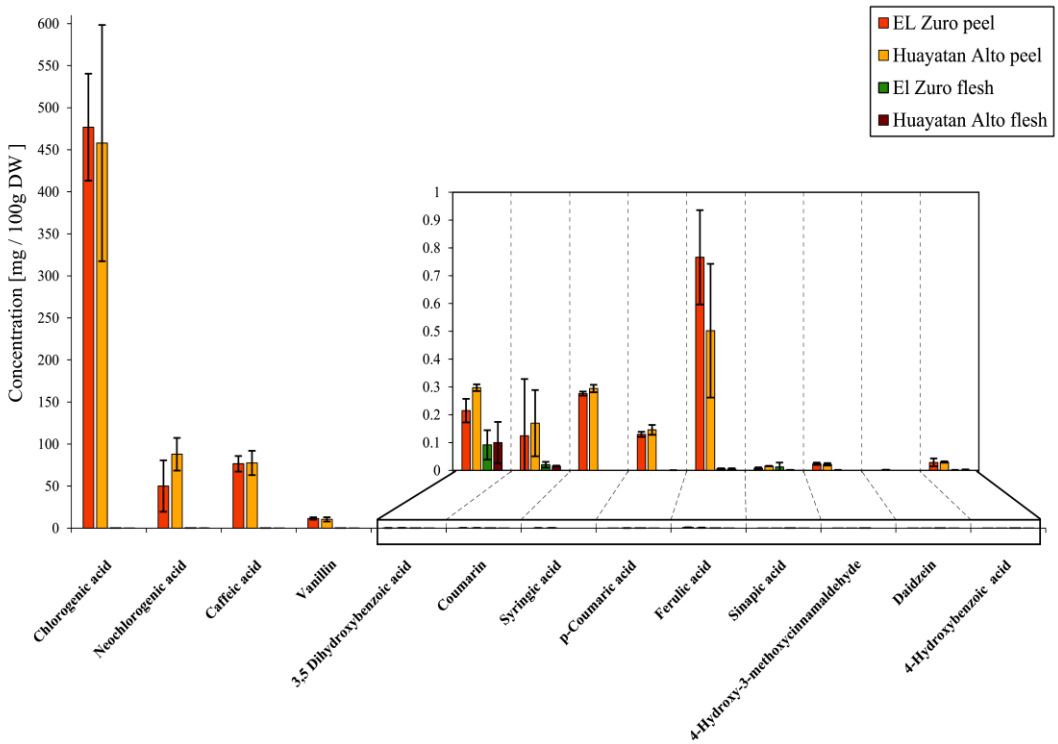

Figure 1. Phenolic compounds in peel and flesh in native Huagalina potato.

Mikami and Yamazawa (2015) studied the protective effects of CGA acid on neuronal cell death induced by glutamate because the release of glutamate during cerebral ischemia triggers the death of neurons, suggesting that this polyphenol protects neurons from glutamate neurotoxicity by controlling calcium ion entry through glutamate receptors. Lirdprapamongkol *et al*., (2005) conducted *in vitro* studies and showed that vanillin has an inhibitory effect on invasion and migration of cancer cells; vanillin *in vivo* showed effects in reducing metastasis of lung tumor colonies compared with controls.

Phenolic compounds found in concentrations less than 1 mg /100 g DW (3,5-dihydroxybenzoic acid, coumarin, syringic acid, p-coumaric acid, ferulic acid, sinapic acid, 4-hydroxy-3-methoxy cinnamaldehyde and daidzein) are also important phytonutrients because potatoes are grown throughout the world and are consumed in large quantities and with fruits and vegetables complete the intake of antioxidants to prevent degenerative diseases (Lewis *et al*., 1999; Ezekiel *et al*., 2013). No genistein and 7-hydroxyflavone were detected, according to the literature both belong to the phenolic group of flavonoids (isoflavones); daidzein is mostly found in soy (Ludueña *et al*., 2007) and recent research has determined that it is also found in quinoa (Lutz *et al*., 2013). The PCA shows a visual description of different groups (Figure 2).

Component F1 shows a clear separation of the phenolic compounds. All of them are mostly located in the peel rather than in the flesh of potatoes grown in EZ and in HA, these results support those reported by other authors (Friedman, 1997; Lewis *et al*., 1999; Ji *et al*., 2012; Yang *et al*., 2016).

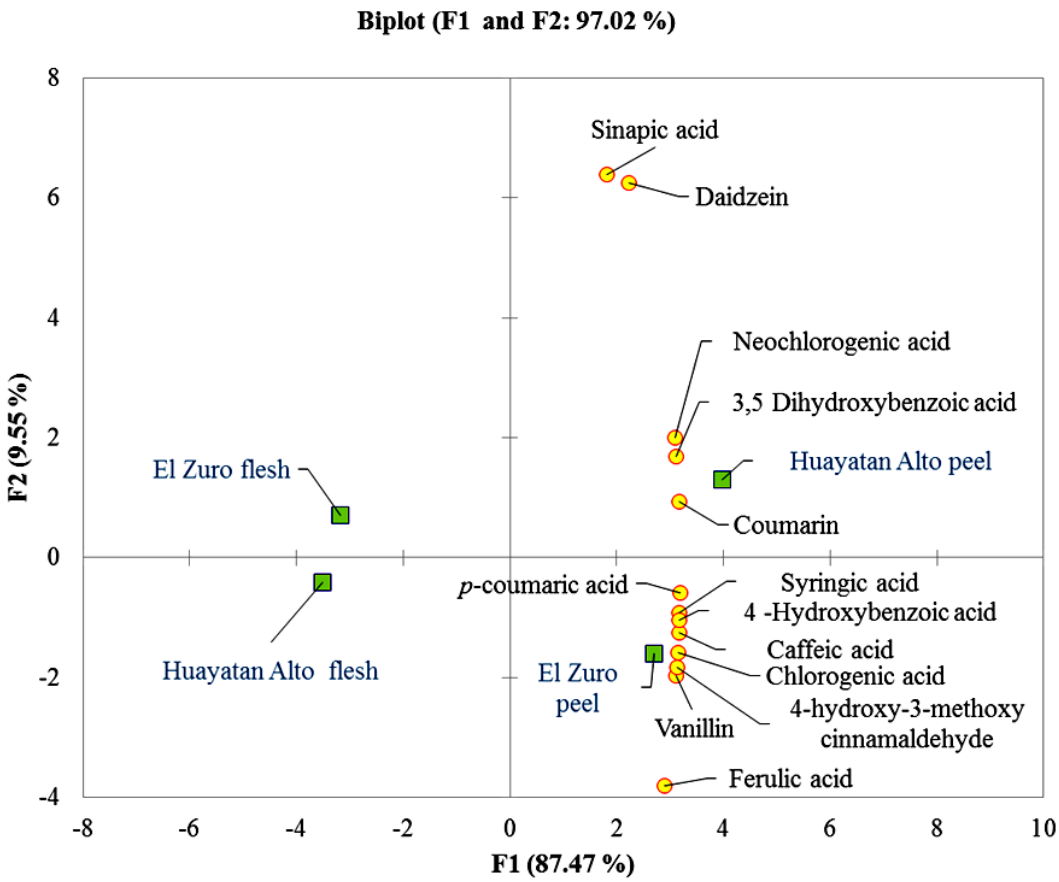

Figure 2. PCA of phenolic compounds in peel and flesh of native Huagalina potato.

With respect to component F2, the peel of potatoes grown in EZ shows 62% of the metabolites studied; syringic acid, 4-hydroxybenzoic and caffeic acid are strongly correlated and highly significant ($r > 0.90$ and $p < 0.05$), in the same way are chlorogenic acid, 4-hydroxy-3-methoxycinnamaldehyde and vanillin.

The difference in phenolic content in crops in EZ and in HA could be explained by environmental differences in field trials, long culture days at low temperatures (Reyes *et al*., 2004), by the use of organic fertilizers and cold environment (Hajslova *et al*., 2005) and cultivation under drought stress (Delgado *et al*., 2001).

Information is still limited on the influence of organic and conventional farming on the content of phytonutrients; it has only been reported that organic farming has a high effect on potato tuber yield (Palmer *et al*., 2013) and the differences in protein content were not statistically significant (Bartova *et al*., 2013). Several researchers (André *et al*., 2009; Hamouz *et al*., 2010; Lachman *et al*., 2009; Reddivari *et al*., 2007; Wegener *et al*., 2009) highlight the prevalence of genotypic variation in environmental conditions.

André *et al*. (2009) examined the expression of thirteen genes involved in the biosynthetic pathway of polyphenols in five Andean potato genotypes using real time RT- PCR. They concluded that polyphenolic profiles are correlated to variations in gene expression profiles and further research is required to know the effect of environmental conditions. This correlation is a prerequisite to support breeding programs aiming to improve the functional value of this crop.

4. Conclusions

The native Huagalina potato contains phytochemicals that may help prevent degenerative diseases. UPLC-MS / MS detected thirteen phenolic compounds. Those in higher concentrations (mg/100 g DW) in El Zuro and Huayatan Alto respectively are: Chlorogenic acid (476.82 ± 63.58 and 457.88 ± 140.46), neochlorogenic acid (50.08 ± 30.53 and 87.90 ± 19.42), caffeic acid (76.50 ± 9.32 and 77.53 ± 14.48) and vanillin (11.52 ± 1.38 and 10.41 ± 2.58). The PCA shows a clear separation of the phenolic compounds, all of them are in the peel. The metabolites found in lower concentration than 1 mg /100 g DW (acid 3,5-dihydroxybenzoic acid, coumarin, syringic acid, *p*-coumaric acid, ferulic acid, sinapic acid,4-hydroxy-3-methoxycinnamaldehyde and daidzein) are also important sources of phytonutrients due to the high diet consumption of potatoes which becomes a good vehicle for antioxidant intake.

In conclusion, the native potato Huagalina have the genes that can be expressed in different biosynthetic pathways of the metabolites found in this research and this could be used for varietal recognition or "fingerprint" of this variety. Further research would be necessary to find out if cooking methods affect phytonutrients content.

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